REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
2006	Open Literature	

4. TITLE AND SUBTITLE

Poly (ADP-ribose) polymerast (PARP) is essential for sulfur mustard-induced DNA damage repair, but has no role in DNA ligase activation

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Bhat, K. R., Benton, B. J., Ray, R.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

US Army Medical Research Institute of Chemical Defense
ATTN: MCMR-CDR-C
3100 Ricketts Point Road
Aberdeen Proving Ground, MD
21010-5400

8. PERFORMING ORGANIZATION REPORT NUMBER

USAMRICD-P04-038

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

US Army Medical Research Institute of Institute of Chemical Defense
ATTN: MCMR-CDZ-P
3100 Ricketts Point Road
Aberdeen Proving Ground, MD
21010-5400

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

Published in Journal of Applied Toxicology, 26, 452-457, 2006.

14. ABSTRACT

See reprint.

15. SUBJECT TERMS

Poly(ADP-ribose) polymerase (PARP), DNA repair, sulfur mustard, DNA ligase I, DNA repair mechanism

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Radharaman Ray
UNLIMITED	UNLIMITED	UNLIMITED	UNLIMITED	6	19b. TELEPHONE NUMBER *(include area code)* 410-436-3074

P04-038

JOURNAL OF APPLIED TOXICOLOGY
J. Appl. Toxicol. 2006; **26**: 452–457
Published online 11 August 2006 in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/jat.1161

Poly (ADP-ribose) polymerase (PARP) is essential for sulfur mustard-induced DNA damage repair, but has no role in DNA ligase activation†

K. Ramachandra Bhat,[2] Betty J. Benton[1] and Radharaman Ray[1,*]

[1] Cell and Molecular Biology Branch, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5400, USA
[2] Department of Chemistry, Lincoln University, Lincoln University PA-19352-0999, USA

Received 1 March 2006; Accepted 24 May 2006

ABSTRACT: Concurrent activation of poly (ADP-ribose) polymerase (PARP) and DNA ligase was observed in cultured human epidermal keratinocytes (HEK) exposed to the DNA alkylating compound sulfur mustard (SM), suggesting that DNA ligase activation could be due to its modification by PARP. Using HEK, intracellular ^{3}H-labeled NAD$^+$ (^{3}H-adenine) was metabolically generated and then these cells were exposed to SM (1 mM). DNA ligase I isolated from these cells was not ^{3}H-labeled, indicating that DNA ligase I is not a substrate for (ADP-ribosyl)ation by PARP. In HEK, when PARP was inhibited by 3-amino benzamide (3-AB, 2 mM), SM-activated DNA ligase had a half-life that was four-fold higher than that observed in the absence of 3-AB. These results suggest that DNA repair requires PARP, and that DNA ligase remains activated until DNA damage repair is complete. The results show that in SM-exposed HEK, DNA ligase I is activated by phosphorylation catalysed by DNA-dependent protein kinase (DNA-PK). Therefore, the role of PARP in DNA repair is other than that of DNA ligase I activation. By using the DNA ligase I phosphorylation assay and decreasing PARP chemically as well as by PARP anti-sense mRNA expression in the cells, it was confirmed that PARP does not modify DNA ligase I. In conclusion, it is proposed that PARP is essential for efficient DNA repair; however, PARP participates in DNA repair by altering the chromosomal structure to make the DNA damage site(s) accessible to the repair enzymes. Published in 2006 by John Wiley & Sons, Ltd.

KEY WORDS: poly(ADP-ribose) polymerase (PARP); DNA repair; sulfur mustard; DNA ligase I; DNA repair mechanism

Introduction

Sulfur mustard (SM, bis-(2-chloroethyl) sulfide) inflicts debilitating injury to skin (blistering or vesication) and to other organs exposed to it. At the molecular level, SM targets chromosomal DNA, RNA and proteins. Following damage by alkylating agents such as dimethyl sulfate (Bhat and Subbarao, 1990) or sulfur mustard, stimulation of poly (ADP-ribose) polymerase (PARP) (Clark and Smith, 1993) and DNA ligase activity (Bhat *et al.*, 1998) has been observed. It has been suggested that in response to DNA damage, DNA ligase is stimulated by (ADP-ribosyl)ation (James and Lehman, 1982; Shall, 1994). SM injury initiates two processes, namely, DNA repair and apoptosis. SM produces alkyl adducts, cross-links and double-strand breaks in the chromosomal DNA (Papirmeister *et al.*, 1991). Repair of this damage can restore DNA integrity and cell viability. On the other hand, apoptosis leads to caspase-3-mediated PARP degradation, DNA fragmentation and cell death.

The repair of SM-induced DNA damage was studied in a model system, cultured human epidermal keratinocytes (HEK). DNA ligase is an essential enzyme for DNA replication and repair. In mammals, three distinct DNA ligases have been identified and characterized (Tomkinson *et al.*, 1991). Among these, the ~120 kDa DNA ligase I has been studied the most. This enzyme is DNA substrate specific and is involved in DNA repair and replication. DNA ligases III and IV have similar substrate specificity in that they can ligate both DNA-DNA and RNA-DNA substrates (Tomkinson *et al.*, 1998). DNA ligase IV is a ~100 kDa enzyme (Wei *et al.*, 1995) and is considered to be specific for V(D)J recombination (Frank *et al.*, 1998), which assembles the variable (V), diversity (D) and joining (J) antigen receptor gene segments within a lymphoid cell, generating a diverse repertoire of receptors in T and B cells. A possible mechanism of DNA ligase stimulation may be through (ADP-ribosyl)ation, as suggested previously (James and Lehman, 1982; Shall, 1994), or through

* Correspondence to: R. Ray, Cell and Molecular Biology Branch, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010-5400, USA.
Disclaimer: The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the US Army or the Department of Defense.
E-mail: radharaman.ray@us.army.mil
† This article is a U.S. Government work and is in the public domain in the U.S.A.
Contract/grant sponsor: Short Term Analytical Service Contract from the US Army Medical Research Institute of Chemical Defense (USAMRICD).

phosphorylation by a kinase. It was reported that the stimulation of DNA ligase I activity in HEK exposed to SM is specific to double-strand breaks in DNA and is mediated by DNA-dependent protein kinase (DNA-PK) (Bhat *et al.*, 1999; Bhat *et al.*, 2006). In addition, it was observed that the half-life of activated DNA ligase is four-fold greater in the presence of 3-amino benzamide (3-AB), a PARP inhibitor (Bhat *et al.*, 1998). The rate constant for repair of SM-damaged DNA is reduced by about three-fold when PARP is inhibited by anti-sense mRNA expression (Bhat *et al.*, 2000), suggesting that PARP is required for efficient DNA repair. If DNA ligase is not activated by (ADP-ribosyl)ation, it is puzzling that PARP inhibition influences DNA ligase activity. A similar observation was made in the presence of an intracellular Ca^{2+} chelator, BAPTA AM (1,2 bis (2 amino phenoxy) methane N, N, N′, N′- tetra acetic acid methyl ester) (Bhat *et al.*, 1998). These observations indicate a need for a better understanding of the role of PARP in the repair of damaged DNA. Furthermore, PARP inhibitors have been suggested as prospective antidotes for SM injury and, therefore, understanding the role of PARP in SM injury is important in developing mechanism-based antidotes for SM injury.

DNA ligase I activation by phosphorylation appears to be a usable biomarker of SM-induced chromosomal DNA damage and the initiation of DNA repair. This biomarker may be useful in monitoring DNA damage and repair associated with a therapeutic approach such as the use of PARP inhibitors in vesicant damage intervention. This report presents evidence that DNA ligase I is not the target of PARP action. We suggest that PARP is essential for efficient DNA repair, and that the PARP action on nuclear proteins such as histones is to unfold the chromatin through poly(ADP-ribosyl)ation and to make chromosomal DNA damage accessible to DNA repair enzymes.

Materials and Methods

Materials

Sulfur mustard (>98% pure) was obtained from the US Army Edgewood Chemical Biological Center (ECBC), Aberdeen Proving Ground, MD, USA. HEK and keratinocyte growth medium (KGM) were purchased from CAMBREX, Walkersville, MD, USA. Oligo dT cellulose and poly dA were purchased from Pharmacia LKB, Piscataway, NJ, USA. Protein assay reagent was the product of Biorad, Richmond, CA, USA. Protease inhibitors and 3-AB were purchased from Sigma-Aldrich, St Louis, MO, USA. All other chemicals were of analytical grade. Monoclonal antibody to bovine DNA ligase I was a gift from Dr Tomas Lindahl, Imperial Cancer Research Fund, UK, and was also purchased from Sigma-Aldrich, St Louis, MO, USA. Radio-labeled (^{33}P) orthophosphoric acid ($^{33}PO_4^{-3}$) was obtained from PerkinElmer-NEN, Boston, MA, USA. The apoptosis inhibitors Z-VAD-FMK (benzyl oxycarbonyl-Val-Ala-Asp-fluoromethylketone) and AC-DEVD-CHO (N-acetyl-Asp-Glu-Val-Asp-CHO (aldehyde)) were purchased from BD Biosciences, San Diego, CA, USA. The CD95 antibody secreting hybridoma (HB-11726) was obtained from American Type Culture Collection (ATCC), Gaithersburg, MD, USA. The dexamethasone inducible PARP (-) human keratinocyte cell line was a gift from Dr Dean Rosenthal and Dr Mark Smulson, Georgetown University School of Medicine, Washington, DC, USA.

Cells

Stock HEK from adult skin of a single donor at passage 2 were cultured in keratinocyte growth medium (KGM) in 5% CO_2 at 37 °C in a humidified atmosphere to almost 100% confluence in 150 cm^2 flasks before exposure to SM and labeling with $^{33}PO_4^{-3}$ (Bhat *et al.*, 1998).

Exposure of HEK to SM in the Presence of $^{33}PO_4^{-3}$ and Inhibitors of PARP and Apoptosis

Monolayer HEK cultures in 150 cm^2 flasks were grown to confluence, the medium was removed by aspiration, the cells were then washed with warm (37 °C) isotonic saline (3 × 25 ml) and exposed to SM as follows. Frozen stock of SM in saline (Broomfield and Gross, 1989) was thawed and vortexed to obtain a solution (4 mM). This stock solution was diluted to 1 mM in the flasks containing the cells using a defined phosphate-free buffered medium (148 mM NaCl, 5.4 mM KCl, 10 mM $NaHCO_3$, 10 mM D-glucose and 25 mM Hepes, pH 7.4; 339 mOsM) containing 0.5 mCi of $^{33}PO_4^{-3}$ (specific activity, 5280 Ci $mmol^{-1}$) in 10 ml. The flasks were incubated for 30 min at room temperature inside the hood and then transferred to a 37 °C cell culture incubator for 2.0 h. The cells were harvested following this incubation. To study the effects of PARP and apoptosis inhibitors, the cells were incubated with either 3-AB or AC-DEVD-CHO (caspase-3 inhibitor) or Z-VAD-FMK (general caspase inhibitor) or CD95 antibody (inhibitor of apoptosis via the CD95 (Fas) receptor pathway) for 30–60 min in the phosphate-free medium before SM exposure.

Cell Harvesting and Preparation of Cell-free Extracts

The flasks were removed from the incubator, the radioisotope-containing medium was removed, and the flasks were washed with ice-cold saline (3 × 25 ml). The cells

were scraped into ice-cold saline and the pooled cell suspension was centrifuged to pellet the cells. The pellet was suspended in a small volume (1–1.5 ml) of an extraction buffer containing the following: 300 mM NaCl, 50 mM Tris.HCl, pH 7.5, 1 mM EDTA, 0.1% Triton X-100 and 10% glycerol and 10 mM in DTT. In addition, the following protease inhibitors were present: phenyl methyl sulfonyl fluoride 1 mM, pepstatin 5 $\mu g\ ml^{-1}$, aprotinin 2 $\mu g\ ml^{-1}$, leupeptin 1.5 $\mu g\ ml^{-1}$ and N-alpha-P-tosyl-L-chloromethyl ketone, 0.5 $\mu g\ ml^{-1}$ (Tomkinson *et al.*, 1991). The suspended cells were disrupted by four freeze-thaw cycles. A clear cell-free extract was obtained following centrifugation of the suspension at 4 °C using a Sorval centrifuge and SS-34 rotor at 5000 rpm for 30 min. The pellet obtained was extracted again with another 1 ml of extraction buffer, and the clear extract obtained after centrifugation as before was combined with the first and stored at −20 °C before further processing.

Analysis and Quantitation of DNA Ligase I Phosphorylation

The experimental procedures have been described and the results show that DNA ligase I is phosphorylated after exposure to SM (Bhat *et al.*, 1999; Bhat *et al.*, 2006). The phosphorylated DNA ligase I was isolated by affinity chromatography using bovine DNA ligase I monoclonal antibody that cross reacts with human DNA ligase I. Under identical conditions, the extent of phosphorylation can be correlated directly to the chromatographic peak area. These criteria were used to evaluate the effects of PARP suppression and apoptosis inhibitors on DNA ligase phosphorylation due to SM exposure.

Briefly, for affinity chromatography, the bovine DNA ligase I monoclonal antibody was immobilized using the Pierce amino link kit (Pierce, Rockford, IL, USA). The column contained approximately 1 mg of the conjugated antibody and was stored in phosphate buffered saline (PBS) containing 0.04% sodium azide. For DNA ligase I binding, 1 ml cell-free extract was absorbed into the column and left to stand at room temperature for 1 h. The column was then washed first with 14 ml of PBS, followed by 8 ml of 1 M NaCl to remove any weakly bound proteins. Extra washes with 1 M NaCl were used to reduce radioactivity to background level before elution. Bound DNA ligase I was eluted with 12 ml of Pierce IgG elution buffer at a flow rate of ~1 ml min^{-1}. Fractions were neutralized with 1 M Tris-HCl, pH 8.0 when needed.

Protein Assay

Protein was assayed using the Biorad protein assay reagent based on the Bradford method and γ-globulin as the standard (Bradford, 1976).

Results and Discussion

Based on the results of our previous studies, it is proposed that PARP might have a role in DNA ligase activation (Bhat *et al.*, 2000). In later studies, it was demonstrated that DNA ligase I activation is by phosphorylation mediated by DNA-PK in response to SM-induced DNA double-strand breaks (Bhat *et al.*, 1999; Bhat *et al.*, 2006). However, the rate of repair is slower when PARP is suppressed by anti-sense expression, and the rate constant for the repair of SM-induced DNA damage in HEK is about one third of that measured when PARP is fully expressed (Bhat *et al.*, 2000). A parallel observation was also made about the decay rate of activated DNA ligase in SM-exposed HEK. It was found that the half-life of the activated DNA ligase was 4.95 h in the presence of 2 mM 3-AB compared with 1.3 h in its absence (Bhat *et al.*, 1998). These observations suggest that PARP is required for rapid repair of SM-induced chromosomal DNA damage. To further establish the role of PARP in SM-induced DNA ligase I activation and DNA damage repair, PARP was specifically eliminated by dexamethasone-induced PARP anti-sense expression in cells (Rosenthal *et al.*, 1995). In these cells, over 90% of PARP was decreased (unpublished results) offering a better specific inhibition compared with 3-AB where non-specific effects could exist due to its metabolites.

A typical elution profile of phosphorylated DNA ligase I from a bovine DNA ligase I monoclonal antibody column is shown in Figure 1. The data were generated by comparing phosphorylation in the presence of $^{33}PO_4^{-3}$ in SM-exposed and unexposed HEK as described in the Methods section. The areas under the curves obtained by area integration using 'PRIZM' software are directly proportional to the level of phosphorylation. Using this

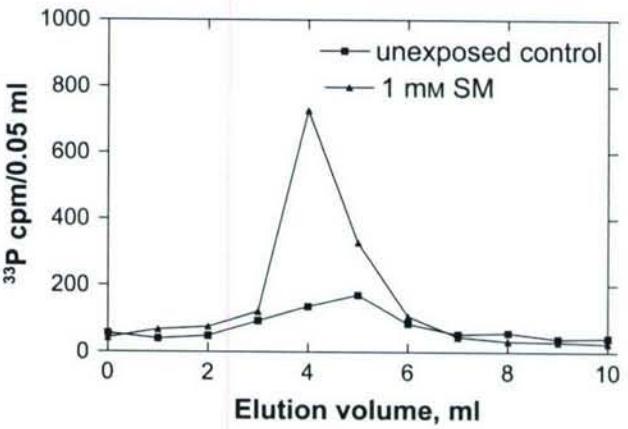

Figure 1. Elution profile of (^{33}P) phosphorylated DNA ligase I in HEK from a monoclonal DNA ligase I antibody affinity column. The extent of phosphorylation is indicated by the area under the curve. The ratio of the areas corresponding to SM-exposed HEK vs SM-unexposed control equals 2

Published in 2006 by John Wiley & Sons, Ltd.

Table 1. Poly(ADP-ribosyl)ation or phosphorylation of DNA ligase I in SM-exposed HEK: effect of decreased PARP activity

Cell type	DNA ligase I modification	Ratio of areas under the curves (SM-exposed/unexposed)
HEK[a] with ^{3}H label in NAD$^+$	Poly(ADP-ribosyl)ation	1.0
HEK (no treatment)	Phosphorylation	2.0
HEK[a] treated with 2 mM 3-AB	Phosphorylation	1.86
PARP (-) HEK[b] cell line	Phosphorylation	1.35

[a] Primary HEK; [b] Dexamethasone inducible PARP (-) cell line.
The data show the ratio (SM-exposed to unexposed controls) of the areas under the curves representing DNA ligase I elution from a bovine DNA ligase I monoclonal antibody affinity column (see Fig. 1). For ^{3}H-NAD$^+$ experiments, the eluted samples corresponding to both SM-exposed and unexposed control showed only background ^{3}H counts without any stimulation due to SM.

method, the ratio obtained for SM-exposed HEK compared with unexposed control was 2. This result indicated a high level of DNA ligase I phosphorylation due to SM exposure in HEK. Using a similar approach, (ADP-ribosyl)ation was examined to determine whether concurrent phosphorylation and poly(ADP-ribosyl)ation is required for DNA ligase I activation.

HEK were used in which NAD$^+$ was metabolically ^{3}H-labeled at adenine (Malanga and Althaus, 1994) and then the cells were exposed to SM. DNA ligase I eluted from the antibody column did not have excess ^{3}H label when compared with the SM-unexposed control cells (Table 1). These results indicate that DNA ligase I is not ADP-ribosylated following SM exposure of HEK. Therefore, phosphorylation is the only modification undergone by DNA ligase I after SM exposure.

Table 1 also shows the effect of decreasing PARP activity in HEK on the extent of DNA ligase I phosphorylation due to SM. In these experiments, PARP was decreased either by using a chemical inhibitor, 3-AB (2 mM), or by expression of dexamethasone inducible PARP anti-sense mRNA in an immortalized HEK cell line (Rosenthal *et al.*, 1995). The ratios of the areas under the respective elution curves of SM-exposed to unexposed cells are given. DNA ligase I phosphorylation in SM-exposed cells was twice as much as in the unexposed control. An enhanced DNA ligase I phosphorylation was also observed in both 3-AB treated cells and PARP (-) cells. The relatively lower level of phosphorylation observed in PARP (-) cells may be attributed to these cells not being primary human epidermal keratinocytes. However, the results suggest that PARP activity is not required for DNA ligase I activation via phosphorylation, and that DNA ligase I is not a target of PARP action during SM-induced chromosomal DNA damage repair.

Caspase-3-catalysed PARP degradation is one of the major events during SM-induced apoptosis. In SM-exposed cells, DNA damage/repair and apoptosis are dependent on SM concentration and time after SM exposure (Bhat *et al.*, 1998; Ray *et al.*, 2005). At high SM concentrations (100 μM or higher), DNA repair and apoptosis are interdependent. It is postulated that DNA repair enhances cell survival, DNA repair inhibition accelerates cell death by apoptosis, and inhibition of apoptosis facilitates DNA repair. If PARP is essential for DNA repair, apoptotic PARP degradation is expected to retard DNA repair. Therefore, inhibition of apoptosis should enhance DNA repair and cell viability.

Table 2. Effect of apoptosis inhibitors on DNA ligase I phosphorylation in SM-exposed HEK

Apoptosis inhibitor added[a]	Ratio of areas under the curve
None	1.0
AC-DEVD-CHO (100 μM)	0.76
Z-VAD-FMK (4 μM)	0.55
CD95 antibody (2 μg ml^{-1})	0.40

[a] Primary HEK.
The extent of ^{33}P incorporation into DNA ligase I (phosphorylation) due to SM in the absence or presence of apoptosis inhibitors was compared. The data show the ratio (apoptosis inhibitor treated vs untreated) of the areas under the curves representing DNA ligase I elution from a bovine DNA ligase I monoclonal antibody affinity column.

The effects of a specific caspase-3 peptide inhibitor, AC-DEVD-CHO (*N*-acetyl-Asp-Glu-Val-Asp-aldehyde), and a general caspase peptide inhibitor, Z-VAD-FMK (benzyloxycarbonyl-Val-Ala-Asp (o-methyl-fluromethyl-ketone)), were tested on the extent of SM-induced DNA ligase I phosphorylation compared with unexposed controls. The extent of phosphorylation was reduced about 24% by the specific caspase-3 inhibitor and about 45% by the general caspase inhibitor (Table 2). In SM-exposed HEK, the apoptotic signal is also transmitted through a cell surface death receptor CD95 (Fas) (Rosenthal *et al.*, 2003). The Fas/FADD (Fas associated death domain) pathway activates caspase-3 processing. Therefore, preventing caspase-3 activation is expected to enhance DNA repair and minimize double-strand break-dependent DNA ligase I phosphorylation. The effects of CD95 (Fas) antibodies on SM-induced DNA damage and DNA ligase I activation were studied. Interestingly, the CD95 antibodies reduced DNA ligase I phosphorylation (about

60%) that requires DNA double-strand breaks (Table 2). Since DNA ligase I phosphorylation is an indicator of DNA damage and possibly DNA repair, these results show that inhibition of apoptosis influences DNA damage and repair.

This report presents data indicating that in SM-exposed HEK, PARP is not involved directly in DNA ligase I phosphorylation and activation (Table 1). However, PARP is necessary for efficient repair of DNA damage due to SM (Bhat *et al.*, 2000).

In apoptosis, activated caspase-3 degrades PARP. Therefore, inhibiting apoptosis in SM-exposed HEK, e.g. by using the general caspase inhibitor Z-VAD-FMK, should prevent PARP degradation. Data presented in Table 2 indicate that in SM-exposed HEK, when apoptosis is inhibited, i.e. PARP is maintained at a higher level, DNA ligase I phosphorylation-cum-activation is reduced. Previously published results from our laboratory showed that DNA ligase I activation via phosphorylation occurs in response to DNA double-strand breaks, and that the level of DNA ligase activity reflects the status of DNA damage and its repair (Bhat *et al.*, 1998; Bhat *et al.*, 1999; Bhat *et al.*, 2006).

An active role of PARP in DNA repair was recently postulated by Sanderson and Lindahl (2002). According to this report, although several enzymes with poly(ADP-ribosyl)ation function have been identified, PARP-1 accounts for over 90% of the total activity detected in mammalian cells following DNA damage. This report also suggested that PARP-1 has the unusual property to act as a 'nick sensor' and binds tightly to DNA single- and double-strand breaks. Upon DNA binding, PARP-1 is activated to (ADP-ribosyl)ate itself, as well as, to a lesser degree, a limited number of other proteins involved in chromatin organization, DNA repair and DNA metabolism. These authors discussed evidence to support the notion that PARP-1 or PARP-1 dependent (ADP-ribosyl)ation might contribute directly or indirectly to efficient DNA repair and the maintenance of genomic stability. In another recent report, d'Erme *et al.* discussed the role of poly(ADP-ribosyl)ation in modulation of chromatin structure (d'Erma *et al.*, 2001). These authors discussed evidence to suggest that the process of poly(ADP-ribosyl)ation is involved in the dynamic transition of chromatin fibers between a condensed and a de-condensed state through the addition of a negative charge to the nuclear proteins. Their results obtained by using scanning force microscopy revealed that poly(ADP-ribosyl)ation of chromatin fiber induces fiber relaxation and de-condensation. Based on these concepts, we propose that in SM-exposed HEK, when apoptosis is inhibited, the higher level of PARP facilitates DNA repair possibly via the mechanisms discussed above, and, therefore, the reduced level of DNA damage is indicated by the decrease in DNA ligase I phosphorylation (Table 2).

We proposed that PARP is essential for the repair of SM-induced chromosomal DNA damage and that may be true for all alkylating chemicals and ionizing radiation that introduce single- and double-strand breaks. Activation of PARP-1 may lead to either activation of DNA repair pathway or apoptotic pathway depending on the cell type and the extent of DNA damage. The switch over from DNA repair to apoptosis is downstream following p53 activation and cell cycle arrest. The protein factors involved and the mechanism of this changeover are not fully understood (Huber *et al.*, 2004). A longer half-life was observed for activated DNA ligase activity following SM exposure in HEK when PARP was inhibited by 3-AB. PARP-1 is the major component in the cell and its inhibition has been shown to decrease double-strand break repair (Veuger *et al.*, 2004). These two observations directly correlate DNA ligase activation with double-strand breaks and their repair and for that, PARP-1 appears to be essential. This observation has led to studies of PARP inhibitors as potential cancer chemotherapeutics (Veuger *et al.*, 2004; Curtin, 2005; Jagtap and Szabo, 2005; Bryant *et al.*, 2005). A recent report has suggested that PARP-1 and XRCC1/DNA ligase III participate in a double-strand repair pathway independent of DNA-PK and XRCC4/DNA ligase IV mediated pathway that may be complementing DNA-PK dependent pathway (Audebert *et al.*, 2004). Overall, our results and conclusions strongly suggest that PARP is essential for double-strand break repair.

Acknowledgements—KRB gratefully acknowledges the financial support through Short Term Analytical Service (STAS) Contracts from the US Army Medical Research Institute of Chemical Defense (USAMRICD) and also for hosting this work in the Cell and Molecular Biology Branch. We thank for Dr Clarence Broomfield and Dr Carmen M. Arroyo of USAMRICD for a thorough review of the manuscript.

References

Audebert M, Salles B, Calsou P. 2004. Involvement of poly(ADP-ribose) polymerase-1 and XRCC1/DNA ligase III in an alternative route for DNA double strand breaks rejoining. *J. Biol. Chem.* **279**: 55117–55126.

Bhat KR, Benton BJ, Ray R. 1998. DNA ligase activation following sulfur mustard exposure in cultured human epidermal keratinocytes. *In Vitro Mol. Toxicol.* **11**: 45–53.

Bhat KR, Benton BJ, Ray R. 2006. DNA ligase I is an *in vivo* substrate for DNA-dependent protein kinase and is activated in response to DNA double strand breaks. *Biochemistry* **45**: 6522–6528.

Bhat KR, Benton BJ, Rosenthal DS, Smulson ME, Ray R. 2000. Role of poly(ADP ribose) polymerase (PARP) in DNA repair in sulfur mustard-exposed normal human epidermal keratinocytes (NHEK). *J. Appl. Toxicol.* **20**: s13–s17.

Bhat KR, Benton BJ, Sparks A, Ray R. 1999. DNA ligase I activation during repair of alkylation damaged DNA is via DNA-dependent protein kinase (DNA-PK) mediated phosphorylation. *FASEB J.* **13**: A1453.

Bhat R, Subbarao SC. 1990. Adenosine diphosphate polymerase (ADPR) polymerase, PARP) and DNA ligase are linked during human DNA repair. *Proc. Am. Assoc. Cancer Res.* **30**: 3.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248–254.

Broomfield CA, Gross CL. 1989. Stability of sulfur mustard in a two-phase storage configuration. *Proc. 1989 Medical Defense Bioscience Review, AD B139550*. US Army Medical Research Institute of Chemical Defense: Aberdeen Proving Ground, MD; 433–436.

Bryant NE, Schultz N, Thomas HD, Parker KM, Flower D, Lopez E, Kyle S, Meuth M, Curtin NJ, Helleday T. 2005. Specific killing of BRCA2 deficient tumors with inhibitors of poly (ADP-ribose) polymerase. *Nature* **434**: 913–917.

Clark O, Smith WJ. 1993. Activation of poly (ADP) ribose polymerase by sulfur mustard in HeLa cell cultures. *Proc. 1993 Medical Defense Bioscience Review, AD A275667*. US Army Medical Research Institute of Chemical Defense: Aberdeen Proving Ground, MD; **1**: 199–205.

Curtin NJ. 2005. PARP inhibitors for cancer therapy. *Expert Rev.* **7**: 1–20.

d'Erma M, Yang G, Sheadley E, Palitti F, Bustamante FC. 2001. Effect of poly(ADP-ribosyl)ation and Mg^{2+} ions on chromatin structure revealed by scanning force microscopy. *Biochemistry* **40**: 10947–10955.

Frank KM, Sekiguchi JM, Seidel KJ, Swat W, Rathburn GA, Cheng H-L, Davidson L, Kangaloo L, Alt FW. 1998. Late embryonic lethality and impaired V(D)J recombination in mice lacking DNA ligase IV. *Nature* **396**: 173–177.

Huber A, Bai P, de Murcia JM, de Murcia G. 2004. PARP-1, PARP-2 and ATM in the DNA damage response: functional synergy in mouse development. *DNA Repair* **3**: 1103–1108.

Jagtap P, Szabo C. 2005. Poly (ADP-ribose) polymerase and the therapeutic effects of its inhibitors. *Nature Rev./Drug Discov.* **4**: 421–440.

James MR, Lehman AR 1982. Role of poly adenosine diphosphate ribose in deoxyribonucleic acid repair in human fibroblasts. *Biochemistry* **21**: 4007–4013.

Malanga M, Althaus FR. 1994. Poly (ADP-ribose) molecules formed during DNA repair *in vivo*. *J. Biol. Chem.* **269**: 17691–17696.

Papirmeister B, Feister AJ, Sabina IR, Ford RD. 1991. Chemistry of sulfur mustard (Chapter 5); Genotoxicity and related cellular effects of sulfur mustard (Chapter 6) In *Medical Defense Against Mustard Gas*. CRC Press: Boca Raton, FL; 91–154.

Ray R, Hauck S, Kramer R, Benton BJ. 2005. A convenient fluorometric method to study sulfur mustard-induced apoptosis in human epidermal keratinocytes monolayer microplate culture. *Drug Chem. Toxicol.* **1**: 105–116.

Rosenthal DS, Shima TB, Celli G, DeLuca LM, Smulson ME. 1995. Engineered human skin model using poly(ADP-ribose) polymerase anti-sense expression shows a reduced response to DNA damage. *J. Invest. Dermatol.* **165**: 38–43.

Rosenthal DS, Velena A, Chou F-P, Schlegel R, Ray R, Benton BJ, Anderson D, Smith WJ, Simbulan-Rosenthal CM. 2003. Expression of dominant-negative Fas-associated death domain blocks human keratinocyte apoptosis and vesication induced by sulfur mustard. *J. Biol. Chem.* **278**: 8531–8540.

Sanderson RJ, Lindahl T. 2002. Down regulation of DNA repair synthesis at DNA single-strand interruptions in poly(ADP-ribose) polymerase-1 deficient murine cell extracts. *DNA Repair* **1**: 547–558.

Shall S. 1994. The function of poly ((ADP-ribosyl)ation) in DNA breakage and rejoining. *Mol. Cell. Biochem.* **138**: 71–75.

Tomkinson AE, Chen J, Besterman J, Husain I. 1998. In *DNA Repair in Higher Eukaryotes*, Nickoloff JA, Hoekstra MF (eds). Humana Press: Totowa, NJ, USA; **2**: 181–198.

Tomkinson AE, Roberts E, Daly G, Totty NF, Lindahl T. 1991. Three distinct DNA ligases in mammalian cells. *J. Biol. Chem.* **266**: 21728–21735.

Veuger SJ, Curtin NJ, Smith GCM, Durkacz BW. 2004. Effects of novel inhibitors of poly(ADP-ribose) polymerase-1 and the DNA dependent protein kinase on enzyme activities and DNA repair. *Oncogene* **23**: 7322–7329.

Wei Y-F, Robins P, Carter K, Caldecott K, Pappin DJC, Yu GL, Wang R-P, Shell BK, Nash RA, Schar P, Barnes DE, Hastelstine WA, Lindahl T. 1995. Molecular cloning and expression of human cDNA encoding a novel DNA ligase IV and DNA ligase III, an enzyme active in DNA repair and recombination. *Mol. Cell. Biol.* **15**: 3206–3216.

Published in 2006 by John Wiley & Sons, Ltd.